

SEQUENCE LISTING

- <110> BASF Aktiengesellschaft
- 5 <120> Electron donor system for enzymes and its use in the biochemical conversion of substrates
- <130> M/40076
- 10 <140>
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- <160> 35
- 15 <170> PatentIn Ver. 2.1
- <210> 1
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<213> Artificial sequence
- 20 <220>
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Oligonucleotide for genomic Bacillus
megaterium DNA
- 25 <400> 1
gtgaaagagg gatcccatga caattaaaga aatgcc 36
- <210> 2
- 30 <211> 38
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Oligonucleotide for genomic Bacillus
megaterium DNA
- 35 <400> 2
gcctcttgga tccttaccca gccacacgt cttttgcg 38
- 40 <210> 3
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- 45 <220>

10031241.011702

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2025-01-01 10:44:10

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15 Oligonucleotide for tag at C terminus

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P450 BM-3 point mutant F87A

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<213> Bacillus megaterium

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1 5 10 15

aat tta ccg tta tta aac aca gat aaa ccg gtt caa gct ttg atg aaa 96

45 Asn Leu Pro Leu Leu Asn Thr Asp Lys Pro Val Gln Ala Leu Met Lys
20 25 30

att gcg gat gaa tta gga gaa atc ttt aaa ttc gag gcg cct ggt cgt 144

64

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5	Val	Thr	Arg	Tyr	Leu	Ser	Ser	Gln	Arg	Leu	Ile	Lys	Glu	Ala	Cys	Asp	
			50					55					60				
	gaa	tca	cgc	ttt	gat	aaa	aac	tta	agt	caa	gcg	ctt	aaa	ttt	gta	cgt	240
	Glu	Ser	Arg	Phe	Asp	Lys	Asn	Leu	Ser	Gln	Ala	Leu	Lys	Phe	Val	Arg	
			65				70					75					
10	gat	ttt	gca	gga	gac	ggg	tta	ttt	aca	agc	tgg	acg	cat	gaa	aaa	aat	288
	Asp	Phe	Ala	Gly	Asp	Gly	Leu	Phe	Thr	Ser	Trp	Thr	His	Glu	Lys	Asn	
			80				85				90					95	
	tgg	aaa	aaa	gcg	cat	aat	atc	tta	ctt	cca	agc	ttc	agt	cag	cag	gca	336
15	Trp	Lys	Lys	Ala	His	Asn	Ile	Leu	Leu	Pro	Ser	Phe	Ser	Gln	Gln	Ala	
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	Met	Lys	Gly	Tyr	His	Ala	Met	Met	Val	Asp	Ile	Ala	Val	Gln	Leu	Val	
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	Gln	Lys	Trp	Glu	Arg	Leu	Asn	Ala	Asp	Glu	His	Ile	Glu	Val	Pro	Glu	
			130					135					140				
25	gac	atg	aca	cgt	tta	acg	ctt	gat	aca	att	ggg	ctt	tgc	ggc	ttt	aac	480
	Asp	Met	Thr	Arg	Leu	Thr	Leu	Asp	Thr	Ile	Gly	Leu	Cys	Gly	Phe	Asn	
			145				150					155					
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30	Tyr	Arg	Phe	Asn	Ser	Phe	Tyr	Arg	Asp	Gln	Pro	His	Pro	Phe	Ile	Thr	
			160				165			170					175		
	agt	atg	gtc	cgt	gca	ctg	gat	gaa	gca	atg	aac	aag	ctg	cag	cga	gca	576
	Ser	Met	Val	Arg	Ala	Leu	Asp	Glu	Ala	Met	Asn	Lys	Leu	Gln	Arg	Ala	
35				180					185					190			
	aat	cca	gac	gac	cca	gct	tat	gat	gaa	aac	aag	cgc	cag	ttt	caa	gaa	624
	Asn	Pro	Asp	Asp	Pro	Ala	Tyr	Asp	Glu	Asn	Lys	Arg	Gln	Phe	Gln	Glu	
			195					200					205				
40	gat	atc	aag	gtg	atg	aac	gac	cta	gta	gat	aaa	att	att	gca	gat	cgc	672
	Asp	Ile	Lys	Val	Met	Asn	Asp	Leu	Val	Asp	Lys	Ile	Ile	Ala	Asp	Arg	
			210				215					220					
	aaa	gca	agc	ggg	gaa	caa	agc	gat	gat	tta	tta	acg	cat	atg	cta	aac	720
45	Lys	Ala	Ser	Gly	Glu	Gln	Ser	Asp	Asp	Leu	Leu	Thr	His	Met	Leu	Asn	
			225				230					235					
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5	Tyr	Gln	Ile	Ile	Thr	Phe	Leu	Ile	Ala	Gly	His	Glu	Thr	Thr	Ser	Gly	
					260					265					270		
	ctt	tta	tca	ttt	gcg	ctg	tat	ttc	tta	gtg	aaa	aat	cca	cat	gta	tta	864
	Leu	Leu	Ser	Phe	Ala	Leu	Tyr	Phe	Leu	Val	Lys	Asn	Pro	His	Val	Leu	
					275				280					285			
10	caa	aaa	gca	gca	gaa	gaa	gca	gca	cga	gtt	cta	gta	gat	cct	ggt	cca	912
	Gln	Lys	Ala	Ala	Glu	Glu	Ala	Ala	Arg	Val	Leu	Val	Asp	Pro	Val	Pro	
			290					295					300				
15	agc	tac	aaa	caa	gtc	aaa	cag	ctt	aaa	tat	gtc	ggc	atg	gtc	tta	aac	960
	Ser	Tyr	Lys	Gln	Val	Lys	Gln	Leu	Lys	Tyr	Val	Gly	Met	Val	Leu	Asn	
		305					310					315					
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20	Glu	Ala	Leu	Arg	Leu	Trp	Pro	Thr	Ala	Pro	Ala	Phe	Ser	Leu	Tyr	Ala	
	320					325					330					335	
	aaa	gaa	gat	acg	gtg	ctt	gga	gga	gaa	tat	cct	tta	gaa	aaa	ggc	gac	1056
	Lys	Glu	Asp	Thr	Val	Leu	Gly	Gly	Glu	Tyr	Pro	Leu	Glu	Lys	Gly	Asp	
					340					345					350		
25	gaa	cta	atg	gtt	ctg	att	cct	cag	ctt	cac	cgt	gat	aaa	aca	att	tgg	1104
	Glu	Leu	Met	Val	Leu	Ile	Pro	Gln	Leu	His	Arg	Asp	Lys	Thr	Ile	Trp	
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	gga	gac	gat	gtg	gaa	gag	ttc	cgt	cca	gag	cgt	ttt	gaa	aat	cca	agt	1152
30	Gly	Asp	Asp	Val	Glu	Glu	Phe	Arg	Pro	Glu	Arg	Phe	Glu	Asn	Pro	Ser	
			370					375					380				
	gcg	att	ccg	cag	cat	gcg	ttt	aaa	ccg	ttt	gga	aac	ggt	cag	cgt	gcg	1200
35	Ala	Ile	Pro	Gln	His	Ala	Phe	Lys	Pro	Phe	Gly	Asn	Gly	Gln	Arg	Ala	
		385						390				395					
	tgt	atc	ggt	cag	cag	ttc	gct	ctt	cat	gaa	gca	acg	ctg	gta	ctt	ggt	1248
	Cys	Ile	Gly	Gln	Gln	Phe	Ala	Leu	His	Glu	Ala	Thr	Leu	Val	Leu	Gly	
	400					405					410					415	
40	atg	atg	cta	aaa	cac	ttt	gac	ttt	gaa	gat	cat	aca	aac	tac	gag	ctg	1296
	Met	Met	Leu	Lys	His	Phe	Asp	Phe	Glu	Asp	His	Thr	Asn	Tyr	Glu	Leu	
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	gat	att	aaa	gaa	act	tta	acg	tta	aaa	cct	gaa	ggc	ttt	gtg	gta	aaa	1344
45	Asp	Ile	Lys	Glu	Thr	Leu	Thr	Leu	Lys	Pro	Glu	Gly	Phe	Val	Val	Lys	
				435					440					445			
	gca	aaa	tcg	aaa	aaa	att	ccg	ctt	ggc	ggt	att	cct	tca	cct	agc	act	1392

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66

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5	Glu	Gln	Ser	Ala	Lys	Lys	Val	Arg	Lys	Lys	Ala	Glu	Asn	Ala	His	Asn	
	465						470						475				
	acg ccg ctg ctt gtg cta tac ggt tca aat atg gga aca gct gaa gga																1488
	Thr	Pro	Leu	Leu	Val	Leu	Tyr	Gly	Ser	Asn	Met	Gly	Thr	Ala	Glu	Gly	
	480						485						490			495	
10	acg gcg cgt gat tta gca gat att gca atg agc aaa gga ttt gca ccg																1536
	Thr	Ala	Arg	Asp	Leu	Ala	Asp	Ile	Ala	Met	Ser	Lys	Gly	Phe	Ala	Pro	
				500						505						510	
15	cag gtc gca acg ctt gat tca cac gcc gga aat ctt ccg cgc gaa gga																1584
	Gln	Val	Ala	Thr	Leu	Asp	Ser	His	Ala	Gly	Asn	Leu	Pro	Arg	Glu	Gly	
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	gct gta tta att gta acg gcg tct tat aac ggt cat ccg cct gat aac																1632
20	Ala	Val	Leu	Ile	Val	Thr	Ala	Ser	Tyr	Asn	Gly	His	Pro	Pro	Asp	Asn	
	530						535						540				
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	Ala	Lys	Gln	Phe	Val	Asp	Trp	Leu	Asp	Gln	Ala	Ser	Ala	Asp	Glu	Val	
	545						550						555				
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	560						565						570			575	
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	Asp	Phe	Glu	Gly	Thr	Tyr	Glu	Glu	Trp	Arg	Glu	His	Met	Trp	Ser	Asp	
	610						615						620				
40	gta gca gcc tac ttt aac ctc gac att gaa aac agt gaa gat aat aaa																1920
	Val	Ala	Ala	Tyr	Phe	Asn	Leu	Asp	Ile	Glu	Asn	Ser	Glu	Asp	Asn	Lys	
	625						630						635				
	tct act ctt tca ctt caa ttt gtc gac agc gcc gcg gat atg ccg ctt																1968
45	Ser	Thr	Leu	Ser	Leu	Gln	Phe	Val	Asp	Ser	Ala	Ala	Asp	Met	Pro	Leu	
	640						645						650			655	
	gcg aaa atg cac ggt gcg ttt tca acg aac gtc gta gca agc aaa gaa																2016

10031241.011702

67

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	ctt	cca	aaa	gaa	gct	tct	tat	caa	gaa	gga	gat	cat	tta	ggg	gtt	att	2112
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10																	
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				705			710					715					
	cta	gat	gca	tca	cag	caa	atc	cgt	ctg	gaa	gca	gaa	gaa	gaa	aaa	tta	2208
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	720					725					730					735	
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	Ala	His	Leu	Pro	Leu	Ala	Lys	Thr	Val	Ser	Val	Glu	Glu	Leu	Leu	Gln	
20					740					745					750		
	tac	gtg	gag	ctt	caa	gat	cct	gtt	acg	cgc	acg	cag	ctt	cgc	gca	atg	2304
	Tyr	Val	Glu	Leu	Gln	Asp	Pro	Val	Thr	Arg	Thr	Gln	Leu	Arg	Ala	Met	
					755				760					765			
25																	
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	Ala	Ala	Lys	Thr	Val	Cys	Pro	Pro	His	Lys	Val	Glu	Leu	Glu	Ala	Leu	
				770				775						780			
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		785					790					795					
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35	800					805					810					815	
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	Glu	Phe	Ile	Ala	Leu	Leu	Pro	Ser	Ile	Arg	Pro	Arg	Tyr	Tyr	Ser	Ile	
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40																	
	tct	tca	tca	cct	cgt	gtc	gat	gaa	aaa	caa	gca	agc	atc	acg	gtc	agc	2544
	Ser	Ser	Ser	Pro	Arg	Val	Asp	Glu	Lys	Gln	Ala	Ser	Ile	Thr	Val	Ser	
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68

	Ala	Ser	Asn	Tyr	Leu	Ala	Glu	Leu	Gln	Glu	Gly	Asp	Thr	Ile	Thr	Cys	
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5	Phe	Ile	Ser	Thr	Pro	Gln	Ser	Glu	Phe	Thr	Leu	Pro	Lys	Asp	Pro	Glu	
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	Thr	Pro	Leu	Ile	Met	Val	Gly	Pro	Gly	Thr	Gly	Val	Ala	Pro	Phe	Arg	
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	Gly	Phe	Val	Gln	Ala	Arg	Lys	Gln	Leu	Lys	Glu	Gln	Gly	Gln	Ser	Leu	
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15	Gly	Glu	Ala	His	Leu	Tyr	Phe	Gly	Cys	Arg	Ser	Pro	His	Glu	Asp	Tyr	
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	ctg	tat	caa	gaa	gag	ctt	gaa	aac	gcc	caa	agc	gaa	ggc	atc	att	acg	2880
	Leu	Tyr	Gln	Glu	Glu	Leu	Glu	Asn	Ala	Gln	Ser	Glu	Gly	Ile	Ile	Thr	
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	960				965					970					975		
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	Gln	His	Val	Met	Glu	Gln	Asp	Gly	Lys	Lys	Leu	Ile	Glu	Leu	Leu	Asp	
				980					985					990			
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30	Gln	Gly	Ala	His	Phe	Tyr	Ile	Cys	Gly	Asp	Gly	Ser	Gln	Met	Ala	Pro	
			995					1000					1005				
	gcc	gtt	gaa	gca	acg	ctt	atg	aaa	agc	tat	gct	gac	gtt	cac	caa	gtg	3072
	Ala	Val	Glu	Ala	Thr	Leu	Met	Lys	Ser	Tyr	Ala	Asp	Val	His	Gln	Val	
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	Ser	Glu	Ala	Asp	Ala	Arg	Leu	Trp	Leu	Gln	Gln	Leu	Glu	Glu	Lys	Gly	
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 Ala Asp Glu Leu Gly Glu Ile Phe Lys Phe Glu Ala Pro Gly Arg Val
 35 40 45
 10 Thr Arg Tyr Leu Ser Ser Gln Arg Leu Ile Lys Glu Ala Cys Asp Glu
 50 55 60
 Ser Arg Phe Asp Lys Asn Leu Ser Gln Ala Leu Lys Phe Val Arg Asp
 15 65 70 75 80
 Phe Ala Gly Asp Gly Leu Phe Thr Ser Trp Thr His Glu Lys Asn Trp
 85 90 95
 Lys Lys Ala His Asn Ile Leu Leu Pro Ser Phe Ser Gln Gln Ala Met
 20 100 105 110
 Lys Gly Tyr His Ala Met Met Val Asp Ile Ala Val Gln Leu Val Gln
 115 120 125
 25 Lys Trp Glu Arg Leu Asn Ala Asp Glu His Ile Glu Val Pro Glu Asp
 130 135 140
 Met Thr Arg Leu Thr Leu Asp Thr Ile Gly Leu Cys Gly Phe Asn Tyr
 145 150 155 160
 30 Arg Phe Asn Ser Phe Tyr Arg Asp Gln Pro His Pro Phe Ile Thr Ser
 165 170 175
 Met Val Arg Ala Leu Asp Glu Ala Met Asn Lys Leu Gln Arg Ala Asn
 180 185 190
 35 Pro Asp Asp Pro Ala Tyr Asp Glu Asn Lys Arg Gln Phe Gln Glu Asp
 195 200 205
 Ile Lys Val Met Asn Asp Leu Val Asp Lys Ile Ile Ala Asp Arg Lys
 210 215 220
 40 Ala Ser Gly Glu Gln Ser Asp Asp Leu Leu Thr His Met Leu Asn Gly
 225 230 235 240
 Lys Asp Pro Glu Thr Gly Glu Pro Leu Asp Asp Glu Asn Ile Arg Tyr
 245 250 255
 45 Gln Ile Ile Thr Phe Leu Ile Ala Gly His Glu Thr Thr Ser Gly Leu
 260 265 270

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Leu Ser Phe Ala Leu Tyr Phe Leu Val Lys Asn Pro His Val Leu Gln
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 5 Lys Ala Ala Glu Glu Ala Ala Arg Val Leu Val Asp Pro Val Pro Ser
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 10 Ala Leu Arg Leu Trp Pro Thr Ala Pro Ala Phe Ser Leu Tyr Ala Lys
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 Asp Asp Val Glu Glu Phe Arg Pro Glu Arg Phe Glu Asn Pro Ser Ala
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 25 Met Leu Lys His Phe Asp Phe Glu Asp His Thr Asn Tyr Glu Leu Asp
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 435 440 445
 30 Lys Ser Lys Lys Ile Pro Leu Gly Gly Ile Pro Ser Pro Ser Thr Glu
 450 455 460
 35 Gln Ser Ala Lys Lys Val Arg Lys Lys Ala Glu Asn Ala His Asn Thr
 465 470 475 480
 Pro Leu Leu Val Leu Tyr Gly Ser Asn Met Gly Thr Ala Glu Gly Thr
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 40 Ala Arg Asp Leu Ala Asp Ile Ala Met Ser Lys Gly Phe Ala Pro Gln
 500 505 510
 Val Ala Thr Leu Asp Ser His Ala Gly Asn Leu Pro Arg Glu Gly Ala
 515 520 525
 45 Val Leu Ile Val Thr Ala Ser Tyr Asn Gly His Pro Pro Asp Asn Ala
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71

Lys Gln Phe Val Asp Trp Leu Asp Gln Ala Ser Ala Asp Glu Val Lys
 545 550 555 560
 Gly Val Arg Tyr Ser Val Phe Gly Cys Gly Asp Lys Asn Trp Ala Thr
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 Thr Tyr Gln Lys Val Pro Ala Phe Ile Asp Glu Thr Leu Ala Ala Lys
 580 585 590
 Gly Ala Glu Asn Ile Ala Asp Arg Gly Glu Ala Asp Ala Ser Asp Asp
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 Phe Glu Gly Thr Tyr Glu Glu Trp Arg Glu His Met Trp Ser Asp Val
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 Ala Ala Tyr Phe Asn Leu Asp Ile Glu Asn Ser Glu Asp Asn Lys Ser
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 Lys Met His Gly Ala Phe Ser Thr Asn Val Val Ala Ser Lys Glu Leu
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 Gln Gln Pro Gly Ser Ala Arg Ser Thr Arg His Leu Glu Ile Glu Leu
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 Pro Lys Glu Ala Ser Tyr Gln Glu Gly Asp His Leu Gly Val Ile Pro
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 Arg Asn Tyr Glu Gly Ile Val Asn Arg Val Thr Ala Arg Phe Gly Leu
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 Asp Ala Ser Gln Gln Ile Arg Leu Glu Ala Glu Glu Glu Lys Leu Ala
 30 725 730 735
 His Leu Pro Leu Ala Lys Thr Val Ser Val Glu Glu Leu Leu Gln Tyr
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 Val Glu Leu Gln Asp Pro Val Thr Arg Thr Gln Leu Arg Ala Met Ala
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825

830

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5 Val Ser Gly Glu Ala Trp Ser Gly Tyr Gly Glu Tyr Lys Gly Ile Ala
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10 Ile Ser Thr Pro Gln Ser Glu Phe Thr Leu Pro Lys Asp Pro Glu Thr
 885 890 895

Pro Leu Ile Met Val Gly Pro Gly Thr Gly Val Ala Pro Phe Arg Gly
 900 905 910

Phe Val Gln Ala Arg Lys Gln Leu Lys Glu Gln Gly Gln Ser Leu Gly
 915 920 925

20 Glu Ala His Leu Tyr Phe Gly Cys Arg Ser Pro His Glu Asp Tyr Leu
 930 935 940

Tyr Gln Glu Glu Leu Glu Asn Ala Gln Ser Glu Gly Ile Ile Thr Leu
 945 950 955 960

25 His Thr Ala Phe Ser Arg Met Pro Asn Gln Pro Lys Thr Tyr Val Gln
 965 970 975

His Val Met Glu Gln Asp Gly Lys Lys Leu Ile Glu Leu Leu Asp Gln
 980 985 990

30 Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro Ala
 995 1000 1005

Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln Val Ser
 1010 1015 1020

35 Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu Lys Gly Arg
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Tyr Ala Lys Asp Val Trp Ala Gly
 1045

40

45

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